Observations on metamorphosing behaviour of *Cybister* larvae for development of control measures during pupal stage[1]

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(*With a text figure*)

Introduction

Several species of aquatic beetles, of the genus, *Cybister* (Family Dytiscidae) are of common occurrence in fish nurseries. Both larvae and adults are active predators, on spawn and of fishes.

Mature larvae, at the end of last instar, pupate in moist earth at the waters' edge of ponds. The easy accessability of *Cybister* beetles at the pupal stage in the vicinity of fish nurseries, makes control measures possible and studies were made on metamorphosing behaviour of the larvae, belonging to five species, namely *Cybister cognatus* Sharp, *C. limbatus* (Fabricius), *C. sugillatus* Erichson, *C. posticus* Aube and *C. tripunctatus asiaticus* Sharp. Among these, *C. tripunctatus* is the commonest and occurs in large numbers.

The term "metamorphosis" is used in a restricted sense in the text, only to denote the changes from last larval instar to the finally formed imago.

Material and Methods

(a) *Material*

Larvae and *Cybister* beetles were collected from fish nurseries and ponds mainly around Bombay. A stock of live material was maintained at the laboratory and were fed on minnows.

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(b) *Methods*

(i) Initially, experiments were conducted to find out the total period of pupation in the soil, once the mature larva encloses itself in the pupal cell. For this purpose, a single freshly collected larvae of a species was kept in a glass aquarium tank, measuring 30 × 23 × 23 cm. In the tank, conditions were made to conform as far as possible to natural conditions. A block of laterite soil, of approximately 22 × 15 × 15 cm size, was placed contiguously to the sidewall over a stone slab of 5 cm thickness kept on the bottom. Water level in the remaining portion of the tank was maintained at this height in order to prevent the desiccation of the clod.

Time and date of entry of the larva into the earth as well as emergence, as an adult, were recorded in order to determine the total period of pupation. The temperature of the water at the time of emergence of the adult was also simultaneously recorded.

Since there were no pupation out of freshly brought last instar *Cybister* larvae in the earth-blocks, except once, the experiments were repeated by introducing into the tank such larvae, which were fed intensively with *Gambusia* and other young fishes. These larvae became progressively dull, and stopped feeding and then readily entered the earthen-blocks. Five observations were made for each species of *Cybister* which were identified later from the imagos that emerged, namely *C. tripunctatus, C. cognatus, C. limbatus, C. sugillatus* and *C. posticus.*

(ii) Since the total periods of pupation varied further laboratory experiments were conducted to find out whether the total period of metamorphosis and therefore the duration of moulting into pupa and imago were constant for each species, from the date of initiation of metamorphosis by interning larvae of different species into artificial pupal cells in the earth-blocks, thereby inducing them to undergo post-larval development.

For this purpose, 20 × 15 × 12 cm size blocks, each, made of laterite soil of dry weight of approximately 1500 g and 500 ml of freshwater, were prepared and kept in enamel trays. Water was poured in the tray at the base of these blocks periodically, to prevent desiccation. In each of these blocks, two crude cells of approximately 3 × 2 cm size, were prepared on either end 10 cm apart.

At a time, two larvae of a particular species which had stopped feeding, were introduced in these cells, closing them thereafter from above, with clay-tablets. Next day, the pupal cells were examined to check whether metamorphosis had been initiated by the larvae, which was indicated by the presence of a secondary earthen cap made by the metamorphosing larva under clay-tablets. This was taken as first day of metamorphosis. Subsequently, observations were made, every 24 hours, by opening the cells in one of these blocks, to examine the

progress of metamorphosis. Thus, the duration of moulting into pupa and imago were determined with reference to larvae of different species.

(iii) Larvae of *C. tripunctatus* being the most predominant in fish nurseries, laboratory experiments were conducted on these larvae, with a view to eradicating different metamorphosing stages infesting the soil above the water level, by using carbon disulphide and formaldehyde as fumigants. In all these experiments, mature larvae, showing no further response for feeding, were introduced in the artificial pupal cells, in the earthen-blocks as described above. The blocks were covered with plastic sheets after injecting the fumigants.

Preliminary experiments were conducted to find out the relationship of different quantities of a fumigant injected, and corresponding period of mortality for different metamorphosing stages, contained in the blocks.

In the experiments, four quantities of one of the fumigants, 2, 4, 6 and 8 ml were injected in four different blocks (covered thereafter with plastic sheets), each containing the metamorphosing stages of one of the five different age groups namely 3, 6, 9 and 18 day-old, at a time. At the end of 24 and 48 hours, the cells in the blocks were opened to examine the effects of increasing doses of the fumigant. These experiments showed that even 2 ml of carbon disulphide was lethal to the insects at the end of 48 hours but formaldehyde had no effect. Thus, in subsequent experiments, varying quantities of carbon disulphide had to be tried for determination of quantities of this fumigant, lethal at the end of 48 hours, for each of the advanced metamorphosing stages (1-18 days stages, and unemerged adults).

However, it was important to find out whether the progress in metamorphosis of a particular stage was arrested during the 48 hours period of fumigation. For this purpose, the blocks (covered with plastic sheets after injection) containing 8 and 17 day-old stages were injected with the quantities of carbon disulphide, lethal at the end of 48 hours. At the end of 24 hours and 48 hours, the cells were opened and 8th and 17th day stages were examined to see whether these had moulted into pupa and adults, respectively, (i.e. 9th and 18th day stages).

Similarly, experiments conducted to find out whether the arresting effect can also be brought about by the disturbance caused by the opening and closing of the cells containing 8th and 17th day stages, showed that moulting did not take place at the end of even 48 hours. Thus, these experiments have shown that in both cases, arresting of metamorphosis took place.

With these preliminary but important observations, experiments were continued by injecting varying quantities of carbon disulphide in the centre of the 20 × 15 × 12 cm size blocks, each with two artificial cells, 10 cm apart, each containing one metamorphosing stage at a

time (i.e. one of the 1-18 day old stages + unemerged adults), with a view to determining the lethal quantities at the end of 48 hours for different stages. Before injecting the fumigant, both cells were opened to ascertain whether the metamorphosing stages in the blocks were in normal state of development. This also served as a coordinate arresting factor of metamorphosis during the period of 48 hours of fumigation.

(iv) Experiments were also conducted with a view to determining the comparative toxicity of two more fumigants, namely ethylene dibromide and ethylene dichloride along with carbon disulphide. For this purpose, a distinct stage common for all the five species, i.e. tenerials of *C. tripunctatus, C. limbatus, C. cognatus, C. sugillatus* and *C. posticus,* were selected as experimental material. At a time, different quantities of one of the fumigants were injected into the blocks, forming five sets. Each block in a set contained two tenerials of one species. Thus, the quantity of each of the three fumigants, lethal at the end of 48 hours for different species, was determined.

Observations

i) *Total pupation period*

The experiments have shown that only larvae, which had stopped feeding readily burrowed into the earth-blocks for metamorphosis after a day of exploration. The total period of confinement in the case of *C. tripunctatus* varied between 19-34 days, and in *C. cognatus* and *C. limbatus,* the period varied between 28 to 32 days. With other two species, *C. sugillatus* and *C. posticus,* the period ranged from 25-32 days. In no case did the larvae penetrate the soil beyond 7-8 cm. Water temperature at the time of emergence of the adults varied from 29-30°C.

ii) *Total period of metamorphosis and the duration of pupal and imago stages in different* **Cybister** *larvae*

The artificially introduced larvae started reshaping the interior of the artificial cells by making the inner facet smooth, and then lay coiled at the bottom, at the end of 24 hours' activity. This behaviour was indicative of their undergoing normal metamorphosis, outwardly manifested by the presence of a secondary cap underneath the clay-tablet, placed over the opening of the artificial cell, immediately after confinement.

Observations at one day intervals subsequently have shown that in the case of *C. tripunctatus* pupa was formed on the 9th day, and the adult on 18th day. In *C. sugillatus* and *C. posticus,* pupa was seen on 12th day and the imago on 24th day. With both *C. cognatus* and

C. limbatus, the pupation took place on 15th day and the moulting into imago on 27th day. In all these cases, the newly formed adults remained in pupal cells for varying number of days before emergence. The larval skin after pupation is stuck into the inner wall of the pupal cell, whereas the pupal exuvium is seen underneath the imago in all the species.

iii) *The lethality of carbon disulphide, at the end of* 48 *hours for different metamorphosing stages of* **C. tripunctatus** (Table 1) (Fig. 1).

Preliminary experiments, to find out whether there was any inverse relationship between increasing doses of the fumigant and decreasing survival periods, showed that at the end of 24 hours of fumigant action there was no mortality with 2, 4, 6 and 8 ml of the fumigant, whereas at the end of 48 hours, mortality occurred invariably with each of these quantities of the fumigant for different metamorphosing stages comprising 3, 6, 9, 12, 15 and 18 day stages showing that this period was necessary for fumigant diffusion. Similar experiments with formaldehyde, another common fumigant, have shown that there was no lethal effect during the entire period of observation.

In experiments with 8th and 17th day stages, contained in the pupal cells, the quantities of carbon disulphide (0.75 and 2.0 m) determined previously as lethal at the end of 48 hours, were injected. At the end of this period, that is on the 10th and 19th days, the dead insects were examined, and it was observed that these had not moulted respectively into pupa and imago, showing that metamorphosis was arrested during the entire period of fumigation.

TABLE 1

QUANTITIES OF CARBON DISULPHIDE LETHAL AT THE END OF 48 HOURS FOR DIFFERENT STAGES DURING METAMORPHOSIS IN CASE OF *Cybister tripunctatus.*

Age in days of metamorphosis-stage	Quantity of the fumigant in ml	Age in days of metamorphosis-stage	Quantity of the fumigant in ml
1	2.0	11	0.75
2	2.0	12	1.0
3	2.0	13	1.0
4	2.0	14	1.0
5	1.5	15	1.5
6	1.0	16	2.0
7	1.0	17	2.0
8	0.75	18	1.0
9	0.5	19 to 35	1.0
10	0.5	Unemerged adults	1.0
		Emerged adult	

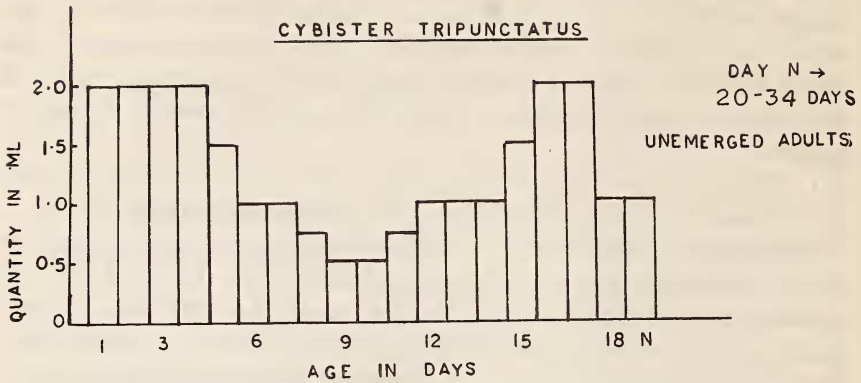

Fig. 1. A graph showing comparative lethal effect of 2 ml of carbon disulphide on various metamorphic stages of *Cybister tripunctatus*.

The other factor, responsible for arresting normal metamorphosis, was the opening and quick closing of the cell-tops. The 8th and 17th day prepupal and preimaginal stages failed to moult respectively into pupa and imago even at the end of 48 hours, showing that such a disturbance retarded the normal pace of metamorphosis.

TABLE 2

COMPARATIVE TOXICITY OF THREE FUMIGANTS IN CASE OF TENERIALS OF FIVE *Cybister* SPECIES, (AT THE END OF 48 HOURS OF FUMIGANT ACTION)

Cybister species	Fumigants	Quantity in ml
1. *Cybister tripunctatus*	Carbon disulphide	1.0
	Ethylene dichloride	0.75
	Ethylene dibromide	0.5
2. *C. cognatus* and 3. *C. limbatus*	Carbon disulphide	0.5
	Ethylene dichloride	0.75
	Ethylene dibromide	0.5
4. *C. sugillatus* and 5. *C. posticus*	Carbon disulphide	0.5
	Ethylene dichloride	0.75
	Ethylene dibromide	0.5

Experiments were conducted, based on preliminary observations, to determine the quantities of carbon disulphide lethal at the end of 48 hours for different stages contained in 20 × 15 × 12 cm size blocks (covered with polythene sheets after fumigant injections). The results are given in Table 1. It can be seen from this Table that 9th and 10th day stages requiring a lethal quantity of only 0.5 ml of the fumigant are the least tolerant and 1-4th and 16-17th day stages requiring 2 ml

of carbon disulphide are the most tolerant. Similar experiments on adults (emerged), confined in the artificial pupal cells in the blocks, have shown that even 1 ml of the fumigant was lethal at the end of 48 hours. These results are illustrated in fig. 1.

iv) *Comparative toxicity of the three fumigants on unemerged imagines of different* **Cybister** *species* (Table 2).

From table 2, it can be seen that the quantities of carbon disulphide, ethylene dichloride and ethylene dibromide lethal for different species were 0.5-1.0, 0.75 and 0.5 ml, respectively, showing that ethylene dibromide is the most toxic and ethylene dichloride, the least, except in case of *C. tripunctatus.*

Discussion and conclusions

Studies on metamorphosing behaviour of different last instar *Cybister* larvae, were made by conducting a series of laboratory experiments, so that a suitable method for their eradication from infested moist soil above water level in fish nurseries could be devised.

Initial experiments were conducted for determining the total periods of confinement in the earth above the water level, after the mature larva constructed a pupal cell for undergoing post-larval development. These experiments were of considerable significance, as different *Cybister* larvae, which could not be identified to their respective species in immature condition could be identified as belonging to five different species from their adults. Amongst the five species of larvae, *Cybister tripunctatus* resembles the larvae of *C. cognatus* and *C. limbatus* except for its size, measuring on an average about 5 cm in length, whereas the larvae of the latter two species are the largest of all the *Cybister* larvae measuring about 7-8 cm in length. Both *C. cognatus* and *C. limbatus* larvae resemble each other except that *C. cognatus* is slightly smaller, and possesses conspicuous longitudinal stripes on the dorsal side. Their adults also appear almost identical except for minor differences in abdominal colour. Larvae of *C. sugillatus* and *C. posticus* also resemble each other, measuring 4.5 cm in length on an average. The larva of *C. posticus* is slightly larger than *C. sugillatus* and possess conspicuous stripes on its dorsal side. The larvae of both species can, however, be distinguished from other *Cybister* larvae, by their distinct black and sclerotized head.

The total period of confinement determined, from the time of last instar larval internment to that of emergence as an adult, varied considerably in case of *C. tripunctatus,* being in the range of 19-43 days, whereas with both *C. cognatus* and *C. limbatus,* the variation was in

the narrow range of only 28-32 days. At the time of emergence of the adults the water temperature was seen to be around 29-30°C.

Considering that the total period of confinement in the earth above the water level are variable, in different *Cybister* larvae, further series of experiments were started to ascertain whether the duration of moulting into pupa and adults were also variable. For this purpose, a technique of inducing the mature larvae to undergo metamorphosis in the artificial cells (with similar dimensions of natural cell, i.e. 2 × 3 cm), prepared in 20 × 15 × 12 cm size earthen blocks, was developed, thus facilitating greatly, further series of experiments. During the metamorphosis period, observations at 24 hour intervals have revealed that *C. tripunctatus* had the shortest period for metamorphosis, the pupa and adult being formed on 9th and 18th day, respectively, whereas with the other two *C. cognatus* and *C. limbatus,* the period was 15 and 27 days respectively. During experimental period water temperature varied around 29-30°C.

These results show that although total period of metamorphosis was constant for each species, the period of confinement of the adult (tenerials), were variable as indicated by the previous series of experiments. Thus, the adult of *C. tripunctatus* remains unemerged for varying periods up to 15 days as against other species which remain quiscent for varying periods up to 8 days.

From the number of days required for the emergence after imago formation, it can be seen that after the pupa moults into adult, it remains in confinement for a minimum period of about 24 hours. Further confinement for varying periods prior to emergence may be dependent upon factors such as temperature.

These observations on metamorphosing behaviour of different mature *Cybister* larvae, thus formed the basis for undertaking further studies on their eradication. For this purpose, *C. tripunctatus* was chosen, as it occurred in large numbers in fish nurseries.

Formaldehyde and carbon disulphide, were selected as fumigants. Formaldehyde is commonly used as soil fumigant in agriculture to control root pests. Similarly, carbon disulphide is a well-known fumigant, used for the purpose since 1925 (Fleming 1926, Gough 1945).

Preliminary experiments have shown that; (1) a period of 48 hours was required for lethal action of different doses of carbon disulphide, possibly due to time taken for initial diffusion of the fumigant vapour through the substance of the block, before concentrating in the cavities of the pupal cells, till mortality occurred; (2) Even 6-8 ml of formaldehyde was not lethal at the end of 48 hours, thus ruling out its use for application in the field and; (3) during the experimental period of 48 hours of fumigation, different stages remained unadvanced in metamorphosis due to two factors, the disturbance caused by opening and

rapid closure of the cell and sublethal concentration of the fumigant vapour in the cell.

With these points in view, different quantities of carbon disulphide were injected in the centre of the blocks (covered subsequently with polythene sheets), containing different metamorphosing stages from 1-8 days and unemerged as well as emerged adults.

These experiments revealed that the quantities of carbon disulphide lethal at the end of 48 hours for different metamorphosing stages, were variable. The 8th and 9th day stages were the least tolerant, even 0.5 ml of the fumigant being lethal, whereas 1-4th and 16-17th day stages were the most tolerant requiring not less than 2 ml of carbon disulphide. The remaining stages required 0.75, 1.0 or 1.5 ml of this fumigant depending upon their capacity of resistance. However, in case of majority of the stages, comprising unemerged as well as emerged adults, 1.0 ml of the fumigant was quite lethal.

The common lethal quantity for all the metamorphosing stages, was 2 ml of carbon disulphide per 20 × 15 × 12 cm size block of laterite soil, containing any of the stages, for bringing about mortality at the end of 48 hours.

A final series of experiments on comparative toxicity of the three fumigants on common stages in metamorphosis, i.e. tenerials of five *Cybister* species, showed that 0.5 ml of ethylene dibromide as well as carbon disulphide were lethal at the end of 48 hours, for all the species except *C. tripunctatus,* which required at least 1 ml of carbon disulphide, whereas for ethylene dichloride, the lethal quantity at the end of 48 hours was 0.75 ml. This indicated that ethylene dibromide is the most toxic and ethylene dichloride, the least.

The use of ethylene dibromide would not only be more effective but also economically feasible. This fumigant again unlike carbon disulphide, has no obnoxious odour and is not inflammable. This chemical has been in use as a soil fumigant in agriculture since 1945 (Shepard 1951).

For application in the field the following recommendation can be made. In fish nurseries the soil above the water line may be marked into units, each corresponding to 20 × 15 × 12 cm size block. Then 2 ml of the fumigant can be conveniently injected in the centre of each of these units (at a depth of nearly 7 cm) with subsequent coverage of the whole strip of 15 cm wide, infested with metamorphosing stages, with plastic sheet or gunny cloth, which can be fastened to the surface of the soil by applying nails along both the edges. Since metamorphosis from last instar larva onwards, in case of *C. tripunctatus* extends upto 18 days with a few additional days for unemerged adults, further fumigation can be done at intervals of about 18 days. In fish nurseries, the whole period of spawn to fry stage would not take more than 18

days even if two crop rotations are taken. Hence, an initial application of the fumigant, prior to stocking of the spawn and a second after about 18 days would be sufficient to check the menace of emerging adults of *C. tripunctatus* in fish nurseries.

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